

بسم الله الرحمن الرحيم

Bovine Brucellosis in El-Huda Area, Al-Gezira State, Sudan

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DEDICATION

Dedicated to my family

To all those who supported me in this study.

To my family.

*Especially my mother, OmelHusien Mohamed
nor and my father.*

Who always prays for me.

To my brothers and sisters.

Who always help me.

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ABSTRACT

The main objective of the present research was to seroprevalence of brucellosis in cattle in Elhuda area, Elmanagil locality, Gezira State. A total of 200 bovine sera were collected randomly from cattle of different age and breeds.

Samples were examined serologically by the Rose Bengal Plate test. Nine samples (4.5%) were positive for anti-*Brucella* antibodies. These positive samples were examined by the serum agglutination test to measure antibody titre/ml which ranged between 20 and 1488iu.

The disease was detected in all age groups (3-17 years) included in the study, but was more prevalent among cows aged 4-8 years old.

It was concluded that bovine brucellosis is of low prevalence in Elhuda area, Gezira State and control measures should be implemented as soon as possible to stop spread of the disease in animals and prevent human infection.

المستخلص

الهدف الاساسي لهذا البحث كان اجراء مسح مصلي لمرض الاجهاض المعدي في الابقار في منطقة الهدى، محلية المناقل ولاية الجزيرة. جمعت 200 عينة سيرم من مختلف الأعمار والسلالات وأجري عليها اختبار الروزبنقال وكانت النتيجة (4.5%) 9 عينات موجبه. كما اجري اختبار تخثر السيرم علي العينات الموجبه للروزبنقال لقياس مستوى الاجسام المضادة فكانت ما بين 20 و1488 وحدة دولية .

ظهر المرض في كل الاعمار (3-17سنة) وكان اكثر انتشاراً في الابقار في عمر 4-8 سنوات.

يمكن الإستنتاج ان البروسيلا موجودة في منطقة الهدى بولاية الجزيرة، ويجب ان تطبق اجراءات السيطرة باسرع ما يمكن لايقاف انتشار المرض في الحيوانات ومنع الانسان من الاصابه.

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INTRODUCTION

Brucellosis is a zoonotic bacterial disease which has a great effect on public and animal health in many countries of the world. It affects a variety of domestic and wild animals and man. It is caused by any one of the members of the genus *Brucella* (Amel, 2005).

According to Krieg and Holt (1984), *Brucella* is a group of bacteria which are morphologically and antigenically similar. It has ten species according to the primary host, *Brucella abortus* (*B. abortus*) (cattle), *B. melitensis* (sheep and goats), *B. suis* (pigs), *B. ovis* (sheep), *B. canis* (dogs) and *B. neotomae* (desert wood rat) (Stonner and Lackman, 1957).

Recently, *B. pinnipedialis* and *B. ceti* a marine strain of *Brucella* (Foster *et al.*, 2007), *B. inopinata* has been isolated from a breast implant infection (Scholz *et al.*, 2009) and *B. microti* Has been isolated from systemically infected common voles (*Microtus arvalis*) in South Moravia (Scholz *et al.*, 2008a). Later on, *B. microti* was isolated from mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) hunted in Austria (Scholz *et al.*, 2008b). Furthermore, specific *B. microti* DNA sequences were recently detected in soil, but whether soil is the primary habitat of *B. microti* remains to be investigated (Scholz *et al.*, 2008c)

The first isolation of *Brucella* organisms from animals was made by Bang (1897). *Brucella melitensis* was the first species reported as the cause of brucellosis due to consumption of raw infected goat milk (Bruce, 1887).

Brucellosis in bovine exhibits one principle symptom i.e. abortion, the first abortion can occur when cow reaches five months of pregnancy. The

majority of abortions are seen around the seventh month, a cow usually aborts once and then becomes a carrier. Some cows may abort a second and occasionally even a third time. The other manifestations occur such as hygroma, orchitis, retention of placenta, weakness of stillbirth, long calving intervals, infertility, bursitis and arthritis, these symptoms occur variably and to a lesser extent in other animal species (Musa *et al.*, 1990b).

Infected cows must be culled if eradication is needed, but this causes economical losses. Milk from infected animals can be treated by pasteurization following international standard efficient methods, so that *Brucella* organisms will be destroyed. To avoid transmission of *Brucella* organisms through the ingestion of infected milk or by the conjunctiva or inhalation or direct skin contact to foetal contents, farmers must be cautious to isolate brucellosis positive animals and also those with symptoms of early delivery or the latent carriers.

Brucella can cross react with *Yersinia enterocolletica* and this can give false positive result, so the antigen was modified by addition of EDTA to make the test more specific (Garin and Trap, 1985).

Brucellosis has a major economic impact due to abortion, consequent decrease in milk yield, death of infected animals and rejection of exported consignments containing infected animals. Also countries incurs cost generated by prophylactic activities, control and eradication programmes, hospitalization of human patients, loss of work or income and failure of financial investment (Chukwu, 1987).

The disease must be controlled by testing, isolation of reactors and Vaccination using full doses of *B. abortus* strain-19 vaccine for calves and reduced dose for adults.

Objectives of the study:

The present study was carried out to:

- 1- Determine seroprevalence of brucellosis in cattle in Elhuda area.
- 2- Measure *B. abortus* antibodies titres of the positive bovine serum samples.

CHAPTER ONE

LITERATURE REVIEW

1.1. Brucellosis

1.1.1 Definition

Brucellosis is a widespread bacterial disease of animals and man caused by any one of the members of the genus *Brucella* (Corbel and Hendry, 1983). It was named brucellosis after David Bruce (1887) who was the first one to isolate the organism and recognized the disease. In animals, the disease is characterized by bacteraemia followed by localization of organisms in the reproductive organs, reticuloendothelial tissues and sometimes joints (Gillespie and Timoney, 1981).

The disease in man is known as Malta fever and is characterized by undulant fever, chills, headache, pains in legs, large joints and lumbar regions, profuse night sweating, insomnia, sometimes laryngitis and bronchitis (Van Der Hoeden, 1964).

1.1.2 Historical background:

Bruce (1887) was the first to isolate *Brucella* from spleens of humans with Malta fever and named it *Micrococcus melitensis*. Mohlor and Tram (1911) isolated *Brucella abortus* from guinea pig inoculated with tonsil material from a child and that was the first instance in which the organism was isolated from a human source. Evans (1918) pointed out that *Micrococcus melitensis* described by Bruce and *Bacillus abortus* isolated by Bang were morphologically and antigenically similar (Young and Corbel, 1989).

The first isolation of *Brucella* organisms from animals was made by Bang (1897), who was the first to report contagious abortion in cattle and other animal species and he named his isolate *Bacillus abortus*, which was followed by other names *Corynebacterium abortus*, *Bacterium abortus* and *Alcaligenes abortus*. Meyer and Shaw (1920) suggested the name *Brucella* for the genus.

In Sudan, animal brucellosis was suspected as early as 1904. The first isolation of *B. abortus* was made by Bennet (1943) from a Frisian herd at Bulgravia dairy farm in Khartoum. However, the first isolation of *B. abortus* from a local breed was from a cow which was aborted at Juba dairy farm (Daffalla, 1962). Haseeb (1950) reported the first case of human brucellosis in Sudan.

1.1.3 Geographic Distribution

Brucellosis is found worldwide but it is well controlled in most developed countries. Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean (OIE, 2004).

Brucella species vary in their geographic distribution. *Brucella abortus* is found worldwide in cattle-raising regions except Japan, Canada, some European countries, Australia, New Zealand and Israel, where it has been eradicated (Ozekic et al., 2003). Eradication from domesticated herds is nearly complete in the U.S. *B. abortus* persists in wildlife hosts in some regions, including the greater Yellowstone area of North America.

Brucella melitensis is particularly common in the Mediterranean. It also occurs in the Middle East and Central Asia around the Arabian Gulf and in some countries of Central America. The organism has been reported

from Africa and India, but it does not seem to be endemic in Northern Europe, Northern America (except Mexico), South East Asia, and Australia. *B. ovis* probably occurs in most sheep raising regions of the world. It has been reported from Australia, New Zealand, North and South America, South Africa and many countries in Europe.

In the past, *B. suis* was found worldwide in swine-raising regions. This organism has been eradicated from domesticated pigs in the U.S, Canada, and many European countries. *Brucella canis* probably occurs of the world; however, New Zealand and Australia appear to be free of this organism. *Brucella* species also seem to be widespread in marine mammal population (OIE; 2004).

1.1.4 Transmission

Brucella abortus, *B. melitensis*, *B. suis* and, *B. canis* are usually transmitted between animals by contact with the placenta, fetus, fetal fluids, and vaginal discharges from an infected animal. Animals are infective after either an abortion or full term parturition. Although ruminants are usually asymptomatic after their first abortion, they can become chronic carriers and continue to shed *Brucella* in milk and uterine discharges during subsequent pregnancies. Dogs may also shed *B. canis* in later pregnancies with or without symptoms. Entry into the body occurs by ingestion and through the mucus membranes, broken skin and possibly intact skin.

Brucella ceti and *B. pinnipedialis*, transmission may occurs by direct contact through mucosa and injured skin, oral route due to ingestion of other infected marine mammals (Foster *et al.*, 2002).

Most or all *Brucella* species are also found in semen. Males can shed these organism for long periods or lifelong. The importance of venereal

transmission varies with the species. It is the primary route of transmission for *B. ovis*, *B. suis*, and *B. canis*.

Brucella species can be spread by fomites, including feed and water. In condition of high humidity, low temperature and no sunlight, these organisms can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes. They can withstand drying, particularly when organic material is present, and can survive in dust and soil, and survive longer when the temperature is low, particularly when it is below freezing (OIE; 2004).

1.1.5 Incubation period:

The incubation period varies with the species and stage of gestation, and often cannot be accurately determined. The length of incubation period was inversely proportional to the stage of fetal development at time of exposure (Thomsen, 1950). The incubation period in brucellosis is affected by several factors such as gestation, exposure, dose, age, vaccination and other unknown host resistance influences (Nicoletti, 1980). In cattle, reproductive losses typically occur during the second half of the pregnancy; thus the incubation period is longer when animals are infected early in gestation. In this species, abortion and stillbirths usually occur two weeks to five months after infection. In pigs, abortions can occur at any time during gestation. In dogs, abortions are most common at approximately 7 to 9 weeks of gestation, but early embryonic deaths have also been reported after 2 to 3 weeks (OIE; 2004).

1.1.6 Epidemiology

Epidemiology of brucellosis varies with the host species affected. For cattle, infection is usually caused by *B.abortus*. However *B. melitensis* and rarely *B.suis* can also establish themselves in cattle. These species are

particularly dangerous to humans. Because of the high virulence of most *B.melitensis* and *B.suis* strains and of the large numbers of bacteria that are excreted by infected animals, *Brucella* is usually transmitted from animal to animal by contact following an abortion. Pasture may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays a little role in the epidemiology of bovine brucellosis. However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection.

In sheep and goats, *B. melitensis* is nearly always the infecting species. *B.ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays greater role. The transmission of the disease is facilitated by purchasing animals from unscreened sources. Swine brucellosis is transmitted by contact with recently aborted sows, by ingestion of contaminated food or exposure to a contaminated environment. However, sexual transmission is particularly important. For all species, embryo transfer is safe provided that recommended procedures are followed. For *B.canis*, sexual transmission is also an important means of spread and males can excrete the organism in large numbers in their semen. Urinary excretion also occurs and is a potential hazard to humans. It should be remembered that dogs can acquire infection with *B. abortus*, *B. melitensis* or *B. suis* from aborted ruminants or swine, usually by ingesting fetal or placental material. In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature

animals; young animals are often resistant. Breed may also affect susceptibility, particularly in sheep. The milking breeds seem to be the most susceptible to *B. melitensis*.

Latent or inapparent infections can occur in all farm animal species. These usually result from infection in utero or in the early post-natal period. Such animals can retain the infection for life and may remain serologically negative until after the first abortion or parturition (WHO, 2006).

The *Brucella* is a facultative intracellular parasite, so it has protection from the innate host defenses and from therapeutic agents. Natural or artificial infections usually persist indefinitely although about 10%- 15% recover spontaneously (Nicoletti, 1980).

1.1.7 Pathogenesis

The establishment and outcome of infection of *Brucella* depend on the number of infecting organisms and their virulence and also on host susceptibility (Price *et al.*, 1990). Bacteria multiply inside phagocytes and disseminate via systemic circulation to other organs or tissues such as the spleen, lymph nodes, uterus, and mammary gland. In males, *B. abortus* can be found mostly in the testicles, where the organisms cause orchitis, and accessory sex glands as well as lymphoid tissue. The bacteremia can last for months, and in cases of chronic disease it can be intermittent, recurring mostly around parturition. The bacteria localize in the uterus during gestation and cause ulceration of the endometrium, the initial lesions are seen in the wall of the uterus, but the organism quickly spreads to the placental cotyledons. Depending on the severity of the lesion, potential sequelae include: abortion, especially in last trimester, stillbirth, and premature or weak calves following abortion or parturition.

Erythritol, a polyhydric alcohol which acts as a growth factor for *Brucellae*, is present in high concentration in the placenta of cattle, sheep, goats and pigs. This growth factor is also found in other organs such as the mammary glands and epididymis, which are targets for *Brucellae*. In chronic brucellosis, organisms may localize in joints and intervertebral discs.

1.1.8 Clinical signs

Brucellosis affects many different organs in animals and consequently the signs of the disease will be influenced by the nature and extent of the infection and the species involved. Some infected animals may not show signs (Bishop *et al.*, 1994).

1.1.8.1 Bovine brucellosis (*B. abortus*)

In cattle, *B. abortus* causes abortion, stillbirths, and weak calves; abortion usually occurs during the second half of gestation. The placenta may be retained and lactation may be decreased. After the first abortion, subsequent pregnancies are generally normal; however, cows may shed the organism in milk and uterine discharges. Epididymitis, orchitis, seminal vesiculitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis. Hygromas, particularly on the leg joints, are common symptoms in some tropical countries. Arthritis can develop after long term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or newborn. Infections in pregnant females are usually asymptomatic.

1.1.8.2 Ovine and caprine brucellosis (*B. melitensis*)

Brucella melitensis mainly causes abortion, stillbirths and the birth of weak offspring; animals that abort may retain the placenta, and milk yield is significantly reduced in animals that abort, as well as in animals whose

udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non pregnant sheep and goats remain asymptomatic.

1.1.8.3 Ovine epididymitis (*B. ovis*)

Brucella ovis affects sheep but not goats. This organism can cause epididymitis, orchitis and impaired fertility in rams; epididymitis may be unilateral or occasionally bilateral. Some rams shed *B. ovis* for long periods without clinically apparent lesions. Abortions, placentitis and prenatal mortality can be seen in ewes but are uncommon.

1.1.8.4 Canine brucellosis

Brucella canis can cause abortions and stillbirth in pregnant dogs. Most abortion occurs late, particularly during the seventh to ninth week of gestation. Usually subclinical although can be severe. Mild fever, emaciation, abortions, arthritis and anestrus (OIE; 2004).

1.1.8.5 Porcine brucellosis (*B. suis*)

In pigs, the most common symptom is abortion, which can occur at any time during gestation, and weak or stillbirth piglets. Swollen joints and tendon sheaths, accompanied by lameness and incoordination.

1.1.8.6 Brucellosis in horses

In horses, *B. abortus* and occasionally *B. suis* can cause inflammation of the supraspinous or supra-atlantal bursa; these syndromes are known, respectively, as fistulous withers or poil evil. In chronic cases, nearby ligaments and the dorsal vertebral spines may become necrotic. *Brucella* associated abortion are rare in horses.

1.1.8.7 Brucellosis in marine mammals

Since 1990, *Brucella* strains have been isolated from a variety of marine mammal species, including seal, dolphins, whale, and other species (Ewalt *et al.*, 1994; Ross *et al.*, 1996; Foster *et al.*, 1996; Clavareau *et al.*, 1998; Wyatt, 1999). These isolates have been classified as *B. ceti* and *B. pinnipedialis*, referring to isolates from cetaceans and seals, respectively (Foster, *et al.*, 2007).

1.1.9 Pathology

A yellowish slimy layer covers the aborted fetus which may be macerated. The afterbirth is edematous, slushy, the affected cotyledons, or part of them are covered by sticky, odorless, a large amount of pathogens is excreted with the evil-smelling, dirty-grey lochia. Microscopically, numerous mononuclear cells and some neutrophils infiltrate the stroma of the chorion. Though fetuses may show no gross changes, petechiae are often to be found in the abomasum and on the mucosa of the bladder of the fetus. In the spleen foci may be present, necrotic foci or microgranulomas in the liver, the lymph nodes, spleen, and kidney can be found microscopically. Gross lesions are not evident on the udder, though the supramammary lymph node may be enlarged, histological interstitial mastitis is evident. An infected bull at first shows an acute febrile general reaction with heavily swollen and painful scrotum, the animal refuses food and is depressed. Acute orchitis is characterized by multifocal or diffuse necrosis of the testicular parenchyma and focal necrotizing epididymitis. Microscopically, the seminal epithelial cells are necrotic and large number of the organisms is present. In the chronic stage spermatic granulomas develop in the testicular parenchyma and epididymitis in response to dead sperm. Hygroma, particularly on the carpal joints, is a characteristic feature of a chronic

infection, but sometimes these hygroma is found on the tarsus as well. Furthermore, the regional lymph nodes and vessels are enlarged (Report, 2001).

1.1.10 Susceptibility to disease and host factor

There are a number of factors which determine the course of the disease, these are:

1.1.10.1 Latent infection

Susceptibility to brucellosis increases with sexual development and pregnancy. Cunningham (1977) found weak and transient antibody titre among heifers exposed to virulent strain of *B. abortus*. Sulieman (1987) showed that calves were least susceptible to infection, while prevalence in lactating cows was the highest among different age groups. Calves may acquire infection in utero or by ingestion of contaminated vaginal discharge or milk. This infection was thought to be temporal, but recent reports showed that a number of heifers calves which were infected at early life, were negative to serological tests, and aborted or had an infected calving during the first pregnancy. These were referred to as latent carriers (Cunningham, 1977). Nagy and Hignett (1976) showed that the neonatal infection led to a degree of immunity against subsequent exposure to infection.

1.1.10.2 Sex

Bulls are more to resistant *Brucella* infection than sexually mature heifers and cows (Nicoletti, 1980). All bulls tested in farms where brucellosis was prevalent gave negative serological tests (Sulieman, 1987). During the acute stage of infection, infected bulls can excrete *Brucella* organisms, but this excretion may cease when the infection becomes chronic (Manthei, 1951), although bulls were not regarded as a major source of

infection. (Bendixen and Blood, 1947) suggested that the disease could be widely spread by infected semen used for artificial insemination.

1.1.10.3 Resistance of host and persistence of infection

The *Brucella* species are intracellular parasites, so they have protection from innate host defenses and their therapeutic agents. Natural or artificial infection usually persists indefinitely although about 10-15% recover spontaneously (Nicoletti, 1980). The effect of heredity on the resistance is not completely known. Resistance to brucellosis could inherit through polygene.

1.1.11 Diagnosis

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk (WHO, 2006). Recently, polymerase chain reaction (PCR) has been shown to be available method for detecting DNA from different fastidious and non cultivated agents (Meyer and Mushuhwar, 1991).

There are many methods which are used for the diagnosis of brucellosis:

1.1.11.1 Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of vaccination programme. It should be noted that all infected materials present a serious hazard and they must be handled with adequate precautions during collection, transport and processing.

1.1.11.1.1 Microscopic examination

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziel-Neelsen(Stamp's) or koster methods (Christofferson and Ottosen, 1941). The presence of large aggregate of intracellular, weakly acid-fast organisms of *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella*.

1.1.11.1.2 Culturing of samples for isolation

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem. *Brucella* can be excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media.

1.1.11.2 Guinea pig inoculation

This method is more successful than the direct culture especially from contaminated material. Injections are made intramuscularly inside the thigh , the guinea pig is killed 4-5 weeks after inoculation and its sera is subjected to five tube agglutination test. Recovery of the organism from the spleen of the guinea pigs or positive SAT at 1/10 or over is taken as evidence of infection (Brinely and Mccullough, 1978).

1.1.11.3 Serological methods

Recently, there are two types of serological tests available; very sensitive ones which are used for screening and definitive ones used for confirmation of infection. As a result, usually more than one type of tests are used for the diagnosis of brucellosis because there is no single test which is both sensitive and specific, has the ability to discriminate between

vaccinated and nonvaccinated animals and could distinguish between antibodies due to infection and those due to cross reaction. Many serological tests were developed for diagnosis of brucellosis using body fluids such as serum, hygroma fluid, milk, vaginal mucus, semen, bursa and muscle juices from suspected cattle; these fluids may contain different quantities of antibodies of the IgG, G1, G2 and other types directed against *Brucella* (Beh, 1974). These tests are Rose Bengal Plate Test (RBPT), Serum and tube agglutination test (SAT or TAT), Complement fixation test (CFT), Card, plate agglutination test, buffered agglutination plate test (BPAT), modified serum agglutination test, anti globulin test (AGT) or Coomb's test, indirect haemolysis test (IHT), haemolysis in gel test (HIGT), enzyme linked immunosorbent assay (ELISA), milk ring test (MRT), whey agglutination test and allergic skin test (AST) (WHO, 1992).

The RBPT, MRT, ELISA and CFT are the conventional serological diagnostic methods and should continue in use for brucellosis surveillance. The important serological tests which are used in diagnosis of brucellosis are:

1.1.11.3.1 Rose Bengal Plate test

This test is widely used as a screening test to detect the presence of *B.abortus* infection in cattle (Morgan *et al.*, 1969); Alton *et al.*, 1975). It can also be used as a definitive test (Nicoletti, 1967). Using antigen stained with Rose Bengal buffered at 3.65 PH to inhibit non-specific agglutinins, but not those of *Brucella* (Rose and Roepke, 1957). Test is a spot agglutination technique, because the test does not need special laboratory facilities and is simple and easy to perform. The test detects specific antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG1 type than IgM and IgG2 types (Levieux, 1974). The

test may yield negative result in infected cattle that give positive result with the CFT (Rose and Roepke, 1957). The low PH (+3.6) of the antigen enhances the specificity of the test. The temperature at which the reaction takes place may influence the sensitivity and specificity of the RBPT (MacMillan, 1990).

1.1.11.3.2 Milk Ring test (MRT)

The MRT is cheap, easy, simple and quick to perform; it detects lacteal anti *Brucella* IgM and IgA bound to milk fat globules. However, it gives false positive when milk contains colostrum, it is at the end of the lactation period, or from cows suffering from a hormonal disorder or from cows with mastitis (Bercovich and Moerman, 1979). Milk that contains low concentrations of lacteal IgM and IgA or which is lacking the fat –clustering factors tests false negative (Keer *et al.*, 1959); Tanwani and Pathak, 1971; Patterson and Deyoe, 1978). Because antibodies decline after abortion or parturition, the reliability of the MRT, using 1ml milk, to detect *Brucella* antibodies in individual cattle or in tank milk is strongly reduced (Hill, 1966). According to WHO report, (1992) the MRT is not suitable for sheep and goats as ring formation does not readily occur. The results are influenced by factors such as mastitis, mechanical agitation and vaccination with *B. abortus* strain 19 vaccine. The test is used to detect brucellosis in dairy cattle but, is not sensitive enough to detect brucellosis in goats (Shimi and Tabatabai, 1981).

1.1.11.3.3 Serum Agglutination test (SAT)

This test is widely used in some countries and its positive result are subjected to the definite CFT. The antigen used in the test is a *Brucella* whole cell and the antibodies detected are those directed against the surface molecules. SAT unlike other tests, detects antibodies of other isotypes

(MacMillan, 1990). It can be performed in tubes or microtitre plates and plate was found to be more sensitive (Heer *et al.*, 1982).

Serum Agglutination test has international standardization; it is used for control programmes and import and export policies (MacMillan and Cockrem, 1985). According to reports of FAO/WHO Export committee on brucellosis (1994), the result of this test in cattle with antibody level less than 30 I.U should be considered negative in non- vaccinated animals or in those with unknown vaccination history. Whereas in the vaccinated over 30 months of age, the level should be more than 30 I.U.

1.1.11.3.4 Complement Fixation test (CFT)

This test is used for confirming the result of the RBPT and SAT. The test was found to be more accurate for bovine brucellosis (Morgan *et al.*, 1973). The CFT detects specific antibodies of the IgM and IgG types that fix complement (Hill, 1963 and Levieux, 1974). Meyer (1979) stated that the test was superior to other test in sensitivity and specificity, and it was found to have the highest specificity in both non- vaccinated and vaccinated cattle when compared with SAT, haemolysis in gel, indirect enzyme immunoassay and buffered plate antigen tests, but is laborious and requires highly trained personnel as well as laboratory facilities. This makes the CFT less suitable for use in developing countries. Although (Corbel, 1972) stated that RBPT and CFT reactions are probable due to the same antibody which is IgG1. Although its specificity is very important for control and eradication of brucellosis it may test false negative when antibodies of the IgG2 type hinder complement fixation (MacMillan, 1990). The CFT measures more antibodies of the IgG1 type than antibodies of the IgM type, as the later are partially destroyed during inactivation. Since antibodies of IgG1 type usually appear after antibodies of the IgM type control and surveillance for

brucellosis is best done with SAT and CFT (Levieux, 1974). Blasco *et al.*, 1994a), found that the CFT was less sensitive than RBPT. Buxton and Fratser (1977) reported that the test useful in detecting chronically infected animals in which the complement fixing antibodies disappear more slowly than agglutinins.

1.1.11.3.5 Anti- globulin (Coomb's) test

The antiglobulin (coomb's) test detects antibodies of the IgG2 type and use to confirm SAT results (Hill, 1963). The coomb's test, although laborious, is particularly important when the SAT is positive and CFT results are negative or conclusive (Kiss, 1971). However Coomb's test results are indicative for infection only when it titres are at least two times than titres of the SAT (Hill, 1963). This test's main limitation, as not all infected cattle show this ratio. The 2-mercatoethanol and the revanol tests detect specific IgG (Rossi and Cantini, 1969), and are usually used to differentiate between infected and vaccinated cattle.

1.1.11.3. 6 Enzyme –Linked ImmunoSorbent Assay (ELISA)

The Enzyme-linked immunosorbent Assay (ELISA) is a highly sensitive method used for serological diagnosis (Sutherland, 1985). The ELISA has proven to be specific and as sensitive as the MRT and SAT in detecting *Brucella* antibodies in milk and semen (Nielsen *et al.*, 1981). ELISA results are usually in agreement with CFT results (Ruppanner *et al.*, 1980); Bercovich and Taaijke, 1990). The test can be used for screening and confirmation of brucellosis in both milk and semen. However, depending on the presence of traces of colostrums in the milk, or the presence of low concentration of lacteal immunoglobulin, ELISA may test false positive or false negative (Bercovich and Taaijke, (1990); Kerkhofs *et al.*, 1990). It seems that the ELISA is less sensitive than the CFT, as some infected cattle

that test positive with CFT may test negative with the ELISA (Cargill, and Clark, 1985; Sutherland, 1984). Some researchers imply that the main advantages of the ELISA when compared with CFT lies its relative simple test procedure (Sutherland *et al.*, 1986). The assay is very costly when a few samples are tested, therefore, it is unsuitable for testing individual animals but it's the ideal test for screening purposes.

1.1.11.3.7 Indirect haemoagglutination test (IHAT)

The test was found useful for the diagnosis of brucellosis in animal and man. It uses LPS of *B. Abortus* or intracellular antigen and could be carried out as a tube or microtitre plate test (Corbel and Dan, 1973). The IHAT is highly sensitive but its specificity was offset by difficulty of interpreting reactions produce at low dilution of sera.

1.1.11.3.8 Allergic Skin test (AST)

It is routinely and officially used for the diagnosis of brucellosis in east European countries (Kolar, 1990). Kolar mentioned that the test could be used in farm animals but it was mainly intended for sheep, goats and pigs. In cattle the test could be used to confirm or current the result of serological test in cattle (Jerabek, 1962). Allergic Skin test is performed strictly into the skin. The side of injection depends on the animal species. The test is specific and does not react to cross reacting organism (Kolar, 1990). Some workers believe that the AST is more sensitive than the serological test (Kolar and Kolarova, 1955).

1.1.11.4 Molecular methods

1.1.11.4.1 Polymerase Chain Reaction (PCR)

The technique is a very useful tool for the diagnosis of brucellosis because of its simplicity, high degree of sensitivity and specificity together with its speed, virility in sample handling and risk reduction for laboratory

personnel (Mortata *et al.*, 2001). Serum sample should be used preferentially over whole blood for the molecular diagnosis of brucellosis (Zerva *et al.*, 2001). The test was used to diagnose brucellosis in goats and it was shown to be more sensitive than the RBPT and culture techniques (Leal-Klevezas *et al.*, 2000).

Recently, Amel (2005) examined 160 bovine milk samples using PCR. She was able to detect *Brucella* DNA from 20 (12.5%) milk samples.

1.2. The genus *Brucella* :

The genus *Brucella* comprises a group of Gram-negative bacteria, which are morphologically and antigenically similar (Evans, 1918). Ten members of the genus are currently known. These are *B. melitensis* (Hughes, 1893), *B. abortus*, *Brucella suis* (Huddleson, 1929), *B. neotomae* (Stonner and Lackman, 1957), *B. ovis* (Buddle, 1956) and recently *B. ceti* and *B. pinnipedialis* a marine strain of *Brucella* (Foster *et al.*, 2007), *B. microti* (Scholz *et al.*, 2008) and *B. inopinata* (Scholz *et al.*, 2009).

1.2.1 Morphology

Member of the genus *Brucella* are cocci, coccobacilli or short rods, measuring 0.5- 0.7µm in diameter and 0.6- 1.5µm in length. The organisms arranged singly and less frequently in pairs, short chain or small groups. They are Gram-negative, non motile and do not form spores and capsule (Krieg and Holt, 1984).

1.2.2 Taxonomy of the genus *Brucella*

The old classification of the genus into ten species *B. melitensis*, *B. abortus*, *B. suis*, *B. neotomae*, *B. ovis* and *B. canis*. (Gorbel and Brinly-Morgan, 1988) is the classical worldwide. *Brucella microti*, *B. ceti*, *B. pinnipedialis* and *B. inopinata*. The first four species are normally observed in the smooth form. Whereas *B. ovis* and *B. canis* have only been

encountered in a rough form. Seven biovars are recognized for *B. abortus* (1- 6 and 9), and 5 for *B. suis* (1-5). However, *B. abortus* biovar 8 no longer exist (Meyer and Morgan, 1973) and *B. abortus* biovar 7 was reported to be mixed culture of *B. abortus* 3-5 (Report, 1986). As a result both biovars were not included in recent classification (Alton *et al.*, 1988) and Corbel, (1990). However, DNA – DNA hybridization studies have shown that only one species *B. melitensis* exist in the genus and other species were actually biovars (Verger *et al.*, 1985). Different species (biovar) have different hosts, sheep and goats are primarily host for the (*B. melitensis* biovar ovis) dogs for (*B. melitensis* biovars canis) and wood rat lepidthomas for (*B. melitensis* biovar *neotomae*) (Corbel, 1990). These species and biovars are differentiated by their host specification, tolerance to basic fuchsin and thionin, CO₂ requirement, rate of urease activity, agglutination with monospecific antisera and susceptibility to *Brucella* phages (Weyant *et al.*, 1996).

1.2.3 Cultural and biochemical characteristics

The *Brucella* are aerobic but some strains require CO₂ for primary isolation. Growth is slow and is usually visible after 48 hours of incubation at 37°C. Colonies are about 0.5mm in diameter and appear round, convex with smooth glistering surface. Enriched media for primary isolation and optimum growth include serum agar, liver infusion, dextrose potato, glycerol potato and *Brucella* agar (Buxton and Fraster, 1977). On blood agar, colonies are usually 0.5-1.0mm in diameter, raised and convex, with an entire edges and smooth shiny surface. Non smooth variants of the other species also occur (Patrick *et al.*, 2003).

1.2.4 Survival of *Brucella* in the environment

Compared with the other non sporing pathogenic bacteria, *Brucella* has relatively high capacity to survive and persist in the environment under suitable conditions. Numerous studies have assessed the persistence of *Brucella* under various environmental conditions, thus when pH>4 , high humidity, low temperature and absence of direct sunlight, *Brucella* may retain infectivity for several months in water, fetal membrane of aborted foeti, faeces and liquid manure, wool, hay, on building, equipments and clothes. *Brucella* are able to withstand drying particularly in the presence of extraneous organic material and remain viable in dust and soil. Survival is prolonged at low temperature; specially bellow 0°C (Alton, 1985; Joint FOW/WHO Committee, 1986; Neicoletti, 1980).The organism is susceptible to an acid PH, disinfectants and direct sunlight. Survive of *Brucella* in milk and dairy products is related to variety of factors including the type and age of product, humidity level, temperature, change in PH and moisture content of storage. *Brucella* does not persist for a long time in ripened fermented cheese. The optimal fermentation time to ensure safety is not known, but is estimated at 3 months (Nicoletti, 1990). However, in normally acidified soft cheese, the strictly lactic acid and short time fermentation and drying increase the survival time of *Brucella*. Previous pasteurization of milk or cream is the only means to ensure safety of these products, the survival time of *Brucella* in meat is short, except in frozen carcasses where the organism can survive for a year.

1.3. Brucellosis in Sudan

Animal brucellosis was suspected as early as 1904. The first case of human brucellosis was confirmed in 1950 by Hasseb. No evidence of

infection was recorded until 1934 when one sample of goat serum of high agglutinating titre was received.

Bennet (1943) reported the disease in a dairy herd in the vicinity of Khartoum and isolated *B. abortus* for the first time.

During the period from 1944- 1952, several reports of abortion from various regions were received from field officers, but serological tests revealed no positive cases.

In 1953 as a result of several cases of undulant fever among European residents in Barakat in Gezira, the milk supplying herds of cattle and flocks sheep and goats kept closely together were serologically tested and found to contain a high percentage of reactors; 50% for sheep, 38% for goat and 26% for cattle and *B. melitensis* was isolated from milk (Dafalla and Khan, 1958).

During the period from late in 1954 and early in 1955, abortions were reported from southern dairy herds, one at Malakal where 75 sera were tested yielding 24 positive and the other at Tonj, Bahr Elgazal province, where 58 sera were tested yielding 9 positive.

In 1956 brucellosis was diagnosed at Joba, Equatoria province dairy farm after storm of abortions. Serological tests revealed about 55% positive reactors in the herd (Dafalla and Khan, 1958).

In 1957, brucellosis was serologically diagnosed in western Sudan both in Elobeid and Nuba mountains and there were 155 serological positive cases (Dafalla and Khan, 1958).

During the year 1958- 1959, samples of sera and milk collected from Nisheshiba and Umbinein revealed 144 positive samples from 1345 bovine sera and 9 out of 104 bovine milk samples. Also at the same period, examination of experimental goats in the Veterinary Research laboratory,

yielded 9 positive out of 313 sera. Examination of 497 goat milk samples with the MRT yielded five positives (Dafalla, 1962).

Elnasri (1960) tested sera collected from cattle in the Upper Nile province.

Abdulla (1966) has surveyed brucellosis in Wadi Halfa District and obtained 3% positive in cattle, 1.7% in sheep and 1.5% in goats.

Mustafa and Nur (1968) investigated brucellosis in Gash and Tokar Districts of Kassala province in Eastern Sudan, showed an incidence of 1.1% and 5.5%, respectively. This was followed by another investigation carried out by Mustafa and Hassan (1969) in which a survey of Kenana cattle of the Fung Districts, Blue Nile province and east of the Blue Nile River, the incidence in eastern and western banks was 8.7% and 5.7%, respectively.

Mustafa and Awad Elkarim (1971) reported the incidence of 1.755 and 5.755 in camels in two districts in Kassala.

Shigidi and Razig (1973) isolated *Brucella abortus* from knee hygroma of a bull.

Ibrahim and Habiballa (1975) investigated the milk collected from twenty-three herds in western Sudan using the MRT. They found that positive MRT reactions varied in different localities. In Western Sudan it ranged between 14.2% to 66.7% from a total of 242 cows, 38% of samples were MRT positive, 41.5% were suspicious and 57.55% were negative. These researchers found that the abortion rate in the two localities was 20.25% and 22.9% in the MRT positive herds and 9.2% and 12.3% in the MRT negative herds, respectively.

Habiballa *et al.*, (1977b) tested 2720 cows in Khartoum Province dairy herds A, B, C and the positive reactors were 0.5% 1.1% and 8.2%, respectively.

In Gezira Province the percentages were 30.9%, 3.1%, 7.1%, and 4.4%, respectively in four dairy herds. And in the Blue Nile province the percentage of positive was 1.6% in one dairy herd and the other herd was negative.

Bakhiet (1981) has studies the incidence of brucellosis in cross-bred and native cattle in private farms in Gezira using SAT and found the percentage of reactors between 1.2% and 22.5% among the native and cross-bred cattle, respectively.

Shallali *et al.*, (1982) examined 124 milk samples from a dairy farm in the Blue Nile province and found 11 samples positive for the MRT.

In 1982 the disease was diagnosed in five out of twenty imported goats kept for breeding in Khartoum province (Osman and Adlan, 1986).

Elwali *et al.*, (1983) tested sera from the Southern Darfur province, using RBPT as screening test and reported 18% positive cases.

Between 1983-and 1986 a total of 14939 sheep sera were collected from Khartoum North quarantine, only 0.275% were positive to complement fixation test, during the same period 1351 sheep sera from Port Sudan quarantine were also found negative (Osman and Adlan, 1986).

Sulieman (1987) investigated prevalence of brucellosis in Khartoum and Gezira province in a total number of 2085 milk and 710 blood samples using SAT, RBPT and MRT, found the prevalence of bovine brucellosis in the two regions 15.2% by MRT and 14.1% by SAT, found no association between infection in dams and daughters all bulls tested react negatively to all blood tests. He inoculated two guinea pigs using MRT positive samples and two isolate *B. abortus* strains which were characterized to be biovars.

Gameel *et al.*, (1987) diagnosed bovine brucellosis in 9 out of 20 dairy herds tested in Khartoum province (Musa *et al.*, 1990b) studied clinical

manifestations of brucellosis in the cattle of the southern Darfur. The authors recommended elimination of cattle with hygroma from the herds. In another study (Musa *et al.*, 1990 a) under took the subject of identification of biovars of *Brucella* species isolated from infected cattle in nomadic, semi- nomadic and sedentary husbandry in Southern Darfur. A total of 1040 heads of cattle were examined and 20% were positive and concluded that brucellosis was wide-spread in the area.

The incidence of brucellosis in camels, in the Sudan was first reported by Mustafa and Awad Elkarim (1971) who reported an incidence of 1.75% and 5.7% in two districts in Kassala province. Abudamir, *et al.*, (1984) reported an overall incidence of *Brucella* antibodies among camels of both sexes from three regions namely Eastern, Central and Western Sudan.

Musa (1995) reported the disease in Darfur states Western Sudan and a prevalence of 13.9% in cattle and 7.76% in camels.

Raga (2000) investigated brucellosis in camels and cattle in Darfur states, Using MRT, RBPT, and SAT and CFT. A total of 904 heads of camels were examined. The prevalence was found to be 6.2%. Hygroma aspirates from knee joints of 10 bulls in Southern Darfour were tested. All samples were found positive for brucellosis.

1.5 Prevention

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them.

The measures of prevention include:

Careful selection of replacement animals, these whether purchased or produced from existing stock, should originate from *Brucella*-free herd or flocks. Pre purchase test are necessary unless the replacements are from

populations in geographically circumscribed area that are known to be free of the disease.

Isolation of replacements for at least 30 days.

Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis.

Herds and flocks should be included in surveillance measures such as periodic Milk Ring Tests in cattle (at least four times per year) and testing of slaughtered animals with simple screening serological procedures, such as the RBPT.

Proper disposal (burial or burring) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.

Cooperation with public health authorities to investigate human cases (WHO, 2006).

1.6. Control and eradication

Brucellosis control and eradication program has been and continues to be multi-faceted. The programme uses:

- Test and isolation/ slaughter.
- Surveillance.
- Quarantine.
- Management.
- Vaccination.

1.6.1 Test and isolation/ slaughter

There are no pathogenic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naïve herds/ flocks is usually a strong indicator of infection. There are serological (and sometimes allergic) tests for identification of possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological

studies. In some cases test and slaughter positive animals are only successful in reducing the incidence if the herd or flock is very low (e.g. 2%). Isolation of animals is essential, especially during and after parturition. The immediate slaughter animals positive for brucellosis is expensive and requires animal owners cooperation. Repeated tests of hers or flock are necessary to reduce the prevalence of brucellosis and to eliminate the disease.

1.6.2 Surveillance

The purpose of surveillance is to identify infected herds not already identified by tracing and investigation of neighbouring properties. It provides assurance that the infection has not spread to other herds in the immediate area. Additional surveillance may be needed to assist the design and implementation of the control strategy. Routine surveillance is usually based on the use of cheap screening tests such as the RBPT. This is then followed with the CFT and ELISA to confirm infection (OIE, 2004).

1.6.3 Quarantine

When a herd has been officially quarantined because of brucellosis, any movement of non- neutered (steers and spayed heifers) cattle into and out of the herd is restricted (steers and spayed heifers). Movement of non-neutered cattle out of quarantined herds is allowed to approve destination only. Approved destinations include quarantined pastures, quarantined feedlot and approved slaughter facilities (OIE, 2004).

1.6.4 Management

As the herd size increase, the probability of infection also increases (Christie, 1969). Sulieman (1987) showed that the prevalence rate increased with increase in herd size. This allows contact with other cows inspite of

hygiene or other control measures (Nicolletti, 1980) also in large herds when infected cattle from outside are introduced in, these may be highly susceptible due to age and non- vaccination. Sulieman (1987) suggested that introducing of such cows in a herd is risky. Among non- vaccinated reactor herds, there is a large effect in reducing infection by use of calving pens. Frequent tests and isolation of infected animals may fail to eliminate the spread of brucellosis were serologically negative cows (O'Hara and Christiansen, 1978).

1.6.5 Vaccination

Effective vaccines have played an important role in reducing the incidence of brucellosis in many countries.

1.6.5.1 *Brucella abortus* strain 19

The most widely used vaccine for the prevention of brucellosis in cattle is prepared from *B. abortus* strain 19. It is an attenuated (live) vaccine and is normally given to female calves aged between three and six months as a single subcutaneous dose of $5-8 \times 10^{10}$ viable organisms. A reduced dose of from 3×10^8 to 3×10^9 organisms can be administered to beef or dairy cattle aged 4-12 months, but 5- 10% of animals will develop persistent antibody titres (Beckett, and MacDiarmid, 1985). Alternatively, the vaccine can be administered to cattle of any age as two doses of $5-10 \times 10^9$ viable organisms given by the conjunctival route; this produces protection without a persistent antibody response (OIE, 2004). *B. abortus* strain 19 is of low virulence for cattle, subcutaneous vaccination of pregnant cattle can result in abortions but this event is rather rare ranging from less than 1% to up to 2.5% under field conditions (Lord *et al.*, 1998).

The presence of LPS with an O-chain strain 19 explains the appearance and persistence of antibodies in serum following administration of this vaccine. These antibodies are detected in the serological assays used for the diagnosis of brucellosis (Corbel, 1989).

1.6.5.2 *B. melitensis* Rev- 1

The live *Brucella melitensis* Rev- 1 strain is considered the best vaccine available for the prophylaxis of brucellosis in small ruminants. The vaccination of pregnant animals with full standard doses of Rev- 1 administered subcutaneously is followed by abortion in most vaccinated animals. The induction of abortions when vaccinating pregnant animals means that there is no entirely safe strategy for Rev- 1 vaccination. Conjunctival vaccination is safer than subcutaneous vaccination but is not safe enough to be applied regardless of the pregnancy status of the animals, and should be used only under restricted conditions, for sheep conjunctival administration of standard doses of Rev-1 during the late lambing season or during lactation is recommended as a whole-flock vaccination strategy (Blasco, 1997). Rev- 1 vaccine was shown to cause human infection and is a risk to human population following secretion of vaccine strain in milk (Elberg, 1995).

1.6.5.3 *Brucella abortus* rough strain RB51

“R” standing for “rough” and “B” for *Brucella*; 51 does not stand for number of passages which were necessary to select strain RB51; it refers to an internal laboratory nomenclature used at the time it was derived. Strain RB51 turned out to be essentially devoid of the O- chain, its roughness being very stable after multiple passages in vitro and in vivo through various species of animals (Bricker and Halling, 1995). RB51 was reported to afford absolute protection to calves and to perform better than S19. Controlled

experiments in calves, however, have shown reduced doses of RB51 to be infective, full doses only partially effective, and RB15, less effective than S19 against severe challenges. Moreover, other observations suggest that RB51 is ineffective when prevalence is high (Moriyon I *et al.*, 2004). Veterinarians and other animal health-care personnel should be made aware of the possible risk for infection associated with the veterinary use of RB51 although evidence of serious disease for humans with a normal immune system has not been officially documented (CDC, 1998).

1.7. Treatment

All *Brucella* strains are sensitive *in vitro* to gentamycin, tetracycline and rifampin. Treatment is likely to be undertaken in animals. Streptomycin, doxycycline and rifampin have become the mainstay in antibiotic therapy for brucellosis (Solera *et al.*, 1997). Combination of doxycycline plus streptomycin is found to be superior to that of doxycycline plus rifampin. The combination with usually doxycycline is necessary, to prevent relapse on antibiotic withdrawal (Maurina and Raoult, 2001). The tetracycline antibiotic is the most effective and inhibits 95% of strains in a concentration of 0.02mg/ml, and is more bactericidal for *Brucella* (Millward *et al.*, 1984). The effectiveness of multiple injections of a combination of a long acting tetracycline (20 mg/kg body weight) was studied by (Millward *et al.*, 1984).

1.8. Economic importance

Animal brucellosis poses a barrier to trade of animals and its products. It could seriously impair socio-economic development; especially for livestock owners (Corbel, 1973). Brucellosis physical and psychological suffering, farmers suffer loss of economic due to abortion, the consequent decrease in milk yield, culling of infected animals, rejection of exported

consignments containing infected animals and prolonged fattening time. The country incurs costs generated by prophylactic activities, control and eradication program, hospitalization of human patient, cost of research, loss of work or income and failure in financial investment (Chukwu, 1987)

1.9. Zoonotic importance

Transmission of brucellosis to humans occurs through the consumption of infected, unpasteurized milk and its products, through direct contact with infected animal parts such as the placenta by inoculation through ruptures of skin and mucous membranes, and through the inhalation of infected aerosolized particles. Brucellosis is an occupational disease in abattoir workers, veterinarians, dairy-industry professionals, and personnel in microbiologic laboratories, one important epidemiologic step in containing brucellosis in the community is the screening of house hold members of infected persons (Imuneef *et al.*, 2004).

Airborne transmission of brucellosis has been studied in the context of using *Brucella* as a biologic weapon. In fact, *B. suis* was the first agent contemplated by the U.S. Army as a potential biologic weapon and is still considered in that category (Smart, 1997). In a hypothetical attack scenario, it was estimated that release of an aerosolized form of *Brucella* under optimal circumstances for dispersion would cause 82,500 cases of brucellosis and 413 fatalities (Kaufmann *et al.*, 1997). Cases of laboratory-acquired brucellosis are the perfect examples of airborne spreading of the disease (Ergonul *et al.*, 2004). After entering the human body and taken up by local tissue, lymph nodes, *Brucella* are transferred through regional lymph nodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system. The period of inoculation usually ranges from two to four weeks. The classic

categorization of brucellosis as acute, sub acute or chronic is subjective and of limited clinical interest. Four species of *brucella* can cause human disease: *B. melitensis*, *B. abortus*, *B. suis* and *B. canis*. Disease from marine species has also emerged (Sohn *et al.*, 2003). The vast majority of cases worldwide are attributed to *B. melitensis* and those caused by *B. abortus* (Dokuzoguz *et al.*, 2005).

Human brucellosis is traditionally described as a disease of protean manifestations. However, fever is invariable and can be spiking and accompanied by rigors, if bacteremia is present, or may be relapsing, mild or protracted. Constitutional symptoms are generally present, physical examination is generally non specific, though lymphadenopathy, hepatomegaly, or splenomegaly is often present, osteoarticular disease is universally the most common complication of brucellosis (Bosilkovski, 2004). The reproductive system is the second most common site of focal brucellosis. Brucellosis can present as epididymo orchitis in men and is often difficult to differentiate from other local disease (Navarro *et al.*, 2001). Brucellosis in pregnancy poses a substantial risk of spontaneous abortion (Khan *et al.*, 2001).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Samples

2.1.1 Sources of samples

A total of 200 serum samples were collected from dairy cattle in Elhuda area Gezira state, during the period between January to February 2009. The animals sampled comprised different types and different age groups.

2.1.2 Collection of samples

Five ml of blood were collected from the jugular vein of each animal, in sterile tubes using disposable syringes. The samples collected were placed in a thermoflask and transported to the laboratory and left to clot. The clots were separated and the tubes were kept overnight at 4°C to separate the serum, then the serum samples separated were placed in sterile tubes and stored at -20° C till used.

2.2 Serological tests

2.2.1 Rose Bengal Plate Test

This test is a simple spot agglutination test using antigen stained with Rose Bengal and buffered to a low PH, usually 3.65 ± 0.05 , this antigen was obtained from Central Veterinary Research Laboratory (CVRL), soba.

The test was performed according to the OIE manual , (2004).

Test procedure

- The serum samples and the antigen were brought at room temperature ($22 \pm 4^{\circ}\text{C}$); only sufficient antigen for the day's tests was removed from the refrigerator.
- An amount of 25-30 μl of each serum sample was placed on a white tile, enamel or plastic plate, or in WHO haemagglutination plate.

- The antigen bottle was shaken well, but gently, and an equal volume of the antigen was placed near each serum spot.
- Immediately after the last drop of antigen has been added to the plate, both the serum and antigen were mixed thoroughly (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2cm in diameter.
- The mixture was rocked gently for 4 minutes at the ambient temperature on a rocker or three directional agitators (if the reaction zone is oval or round, respectively).
- Agglutination was immediately read after the 4 minutes period had completed. Any visible reaction was considered positive. A control serum that gives a minimum positive reaction should be tested before each day's tests are begun to verify the sensitivity of test conditions.

2.2.2 Serum agglutination test

The test was carried out according to Alton *et al* (1975).

Materials

- Antigen: *B. abortus* strain 19 is usually used in preparing the antigen. Other smooth strain of *B. melitensis* and *B. suis* may serve equally well. But only strains of proved agglutination ability should be used. This strain was propagated by culturing in potato agar.
- Glass tube (8mmX 50mm) with rim and metal agglutination boxes for carrying the tubes.
- Automatic pipette and tips.
- Phenol saline.
- Flasks.
- Tested serum samples.

Test procedure

- The antigen was diluted by mixing 1ml of antigen with 9 ml of phenol saline (1/10).
- 0.8ml of phenol saline was placed in the first tube and 0.5 in each succeeding tube.
- 0.2ml of serum under test was transferred to the first tube and mixed thoroughly with the phenol saline already there.
- 0.5 ml of the mixture was carried soon over the second tube. This process continued until the last tube, from which after mixing, 0.5ml of dilution was discarded. This process of doubling dilution resulted in 0.5ml of dilution 1:5, 1:10, 1:20 and soon in each tube.
- To each tube 0.5ml of diluted SAT antigen was then added at the recommended dilution and the contents of the tube were thoroughly mixed, thus giving final serum dilution of 1:10, 1:20, etc...

The tubes were then incubated at 37°C for 20 hours before the results are read.

Interpretation of results

The degree of agglutination was assessed by the amount of clearing that has taken place in the tube as compared with a standard tube. The tubes were examined, without being shaken, against a black background, with a source of light coming from above and behind the tubes. Complete agglutination and sedimentation with water clear supernatant was recorded as +++++, nearly complete agglutination and 75% clearing as +++, marking agglutination and 50% clearing as +, and no clearing as standards were prepared at the time the tests were done and incubated with them.

CHAPTER THREE

RESULTS

Nine (4.5%) samples out of the 200 bovine serum samples examined for brucellosis were found positive with for the RBPT.

The nine positive samples examined by the SAT had antibody titre ranged between 20iu- 1488iu/ml.

Table I. The different age groups examined and showing positive cases

Age	No. examined	Frequency	Positive
≤ 4 years	75	37.5	2
$> 4 \leq 8$ years	104	52.0	6
$\geq 8 \leq 12$ years	18	09.0	1
> 12 years	3	01.5	0
Total	200	100.0	9

Table II. Result of Rose Bengal plate test

	No. examined	Frequency
Negative	191	95.5%
Positive	9	4.5%
Total	200	100.0

Table III. Prevalence rate of brucellosis in relation to age

Age	NO. examined	percentage
≤ 4 years	75	2.67
> 4 ≥ 8 years	104	5.77
≥ 8 ≤ 12 years	18	5.56
> 12 years	3	0.00

Table IV. Antibody titre of brucellosis using SAT

Number of samples	Final dilution of serum	IU/ml
1	1/10	20
2	1/10	23
3	1/20	40
4	Not tested	Not tested
5	1/20	47
6	1/20	47
7	1/40	93
8	1/80	160
9	1/640	1488
Total	8	8

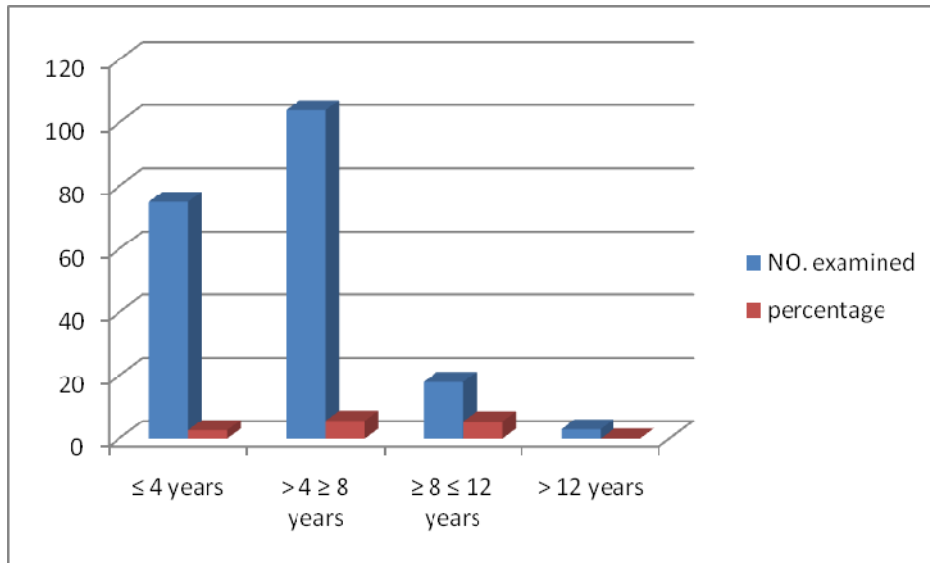


Fig.3: Prevalence of brucellosis in relation to age

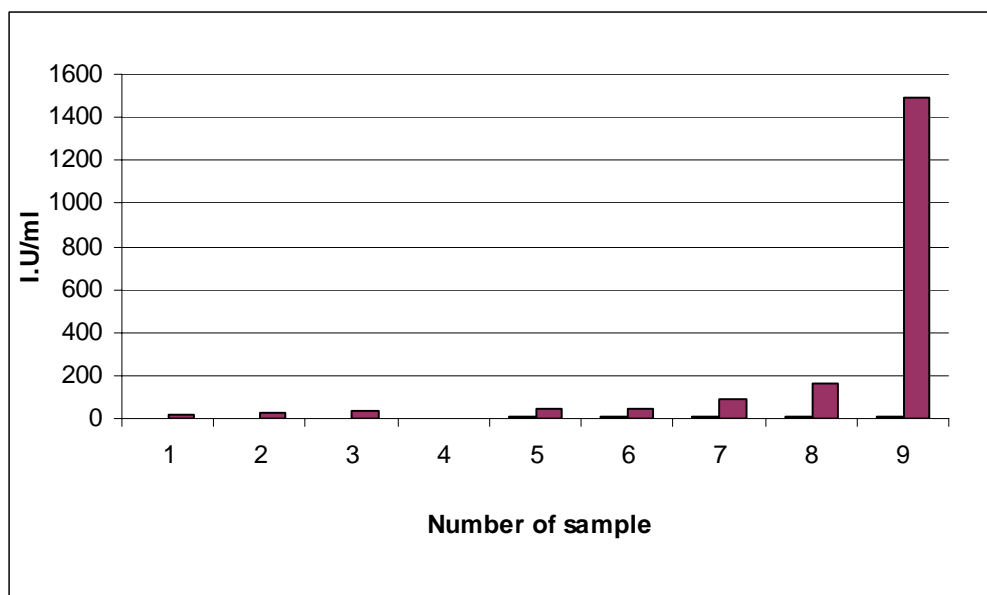


Fig.4: SAT antibody titres of animals positive for brucellosis

CHAPTER FOUR

DISCUSSION

Many surveys were carried out on brucellosis in the Sudan since the diagnosis of the disease by Bennett, (1943), to determine the prevalence of the disease in different parts of the country. The disease serious economical problems in domestic animals such as abortion and decrease in meat, milk, and wool production, causing long calving intervals and infertility. Most of the work was directed towards bovine brucellosis because of the larger numbers and higher value of cattle.

This study was plan to investigate brucellosis in cattle in Elhuda area. The results revealed the prevalence of bovine brucellosis in the area based on the RBPT. This lower prevalence might be attributed to the open system of animal husbandry practiced in the area which reduces the chance of contamination.

Two serological tests, SAT and RBPT were used. Serum samples were examined by RBOT as a screening test and the positive samples were examined by SAT. The results obtained by RBPT also positive by SAT.

It well known that serological diagnosis of bovine brucellosis is considered to be unreliable when applied during the period of 2-3 weeks after abortion or calving (Radostits *et al.*, 2000). The authors explained that low specificity of RBPT against brucellosis in both adult and calves is most likely due to vaccination and colostrums resulting in false positive due to antibody activity from vaccination as well as colostral antibody in calves. On the other hand, false negative reaction for RBPT is attributed to early incubation of the disease and immediately after abortion. The RBPT is very sensitive. However, like all other serological tests, it could sometimes give a

positive result due to S19 vaccination or due to false positive serological reactions (FPSR). Therefore positive reactions should be investigated using suitable confirmatory strategies (including the performance of other tests and epidemiological investigation). False positive reactions occur rarely. Mostly due to prozoning and can sometimes be detected diluting the serum sample or resulting after given time. Nevertheless RBPT appears to be adequate as screening test for detecting infected herds or to guarantee the absence of infection in brucellosis-free herds. Furthermore, (Radositis, *et al.*, 2000) stated that the major problem in brucellosis eradication programs is the false positive reactors.

The results of the study that revealed the disease occurs in in all age groups of cattle. This finding agreed with that of Enright, (1990), who reported that brucellosis occurred in cattle of all ages.

The positive reactors among age groups (Table 1) was found high among cows > 4 years and < 8 years and this could be due the fact that, susceptibility of brucellosis increase with sexual development and pregnancy (Cunningham, 1977), positive reactors among cows >12 year and this may be due to the less number of samples collected from old animals.

The study also revealed that there was high serum antibody titres among >4 year (1488 iu).

The results of 9 positive (4.5%) out of 200 tested animals is low compared with prevalence rates reported by Dafalla, (1962) who reported 10.7% prevalence in Nisheshiba in Gezira State ,Central Sudan, compared with the prevalence reported by (Fayza *et al.*, 1990) who had reported 15.73% prevalence in Khartoum state, and with that reported by Musa (1995), 13.7% in Darfur the result of the study were higher compared with

the prevalence reported by Bakhiet (1981) who has reported 1.2% prevalence of brucellosis in native cattle in private farms in Gezira using SAT, but 22.5% in cross-bred cattle.

CONCLUSION:

It was concluded that the prevalence of bovine brucellosis in Elhuda area is not high.

RECOMMENDATIONS:

It was recommended that:

- 1- More research is needed on isolation and identification of *Brucella* species and biovars which affect animals in Elhuda area.
- 2- Control programmes should be started to stop further spread of brucellosis in animals and man.
- 3- Vaccination of healthy cattle and culling infected ones to control and eradicate of bovine brucellosis in the studied area.
- 4- Education of animal owners and abattoir workers is essential to increase their awareness to avoid their infection and prevent their animals from the disease and the environment from contamination.

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